The NUP214-ABL1 fusion gene in T-cell acute lymphoblastic leukemia (T-ALL) has recently been identified as a possible target for imatinib and related tyrosine kinase inhibitors, but exact data regarding the prognostic impact and frequency of the several putative NUP214-ABL1 mRNA transcripts are still missing. We investigated 279 adult patients with T-ALL treated within the framework of the GMALL 5/93 and 6/99 therapy trials for NUP214-ABL1 by using a novel multiplex real-time, quantitative polymerase chain reaction (PCR). Eleven (3.9%) patients were NUP214-ABL1 positive, and 5 different transcripts were observed; 8 patients had a thymic immunophenotype, 1 had an early T-cell immunophenotype, and 2 had a mature T-cell immunophenotype. NUP214-ABL1-positive and –negative patients did not differ significantly in their major clinical features. In contrast to previous reports suggesting an adverse clinical course for NUP214-ABL1-positive patients, no significant difference in overall survival was observed. Based on the results, we have established and tested a novel PCR method for simplified detection of the NUP214-ABL1 fusion gene. (Blood. 2006;108:3556-3559)

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CD7+, CD5+, CD2+, CD1a+, CD4+/-, CD8+/-, sCD3+/-), or mature T cell (cyCD3+, CD7+, CD5+, CD2+, CD1a+, CD4+/-, CD8+/-, sCD3+).  

RNA was isolated using the TRizol method (Invitrogen, Karlsruhe, Germany) or the PureScript method (Biozym Diagnostik, Hessisch-Oldendorf, Germany). One microgram total RNA was reverse transcribed using the cDNA reaction mix. The quality of the cDNA was confirmed by real-time PCR amplification. RNA from the cell lines H9262 (Oldendorf, Germany) or the PureScript method (Biozym Diagnostik, Hessisch-Oldendorf, Germany) was isolated using the TRIzol method (Invitrogen, Karlsruhe, Germany). One microgram total RNA was reverse transcribed using the HotStarTaq conventional PCR to identify the exact transcript. The primers described were used in separate PCR reactions performed with the HotStarTaq Advantage PCR kit (Qiagen, Hilden, Germany) and the following cycler program: 15 minutes at 95°C, 35 cycles of 20 seconds at 95°C, 20 seconds at 58°C, 20 seconds 72°C. Standard techniques were used to sequence purified PCR products on an ABI sequencer with the respective PCR primers as sequencing primers.  

Real-time quantitative PCR for TLX1 (HOX11) and TLX3 (HOX11L2) 

Ablamont TLX1 or TLX3 expression was detected by real-time PCR in a TaqMan format using the following primers and probes for TLX1 and TLX3, respectively (5'–3'): TLX1-F GATGGAGAGTAACCCGGAGATACAC, TLX1-R TTGCGCGCTTCTCCTTCTT, HOX11L-F FAM-AM AGGACAG-GTTCCAGGTCAACCCCTATCAGA-BHQ1, HOX11L-R CAAGACT-GGTTCACAGGTTATCATCTGGTGACAA, and HOX11L2-F FAM-CAGCTGAAACAGCGCCTTCCAC-BHQ1. PCR conditions were as described. RNA from the cell lines ALL-SIL and HPB-ALL were used as positive controls.  

Results 

Altogether 279 adult T-ALL samples were analyzed, as described. Eleven (3.9%) samples were found to be NUP214-ABL1 positive by real-time quantitative PCR (Table 1).  

Immunologic and genetic characteristics of the NUP214-ABL1–positive patients 

Eight of these patients had a thymic immunophenotype, 1 had an early T-cell immunophenotype, and 2 had a mature T-cell immunophenotype. No particular immunophenotypic features distinguished NUP214-ABL1–positive patients from NUP214-ABL1–negative ones. Cytogenetic data, available for 8 patients, mostly showed a normal karyotype. Eight of 10 NUP214-ABL1–positive patients had evidence of an aberrant expression of HOX11 genes.
were located in occasionally been described (both exons are in the same reading frame).

15-18, multiplex PCR with H2O (lane 15), regulatory domain is encoded by ABL1 all possible NUP214-ABL1 constructed in 10 different gene is not yet well characterized. Thus, forward primers were

Figure 2. Probability of overall survival of NUP214-ABL1–positive and –negative patients. No significant differences were observed in overall survival between NUP214-ABL1–positive (dashed line) and –negative patients (solid line). Table 1 lists the detailed clinical characteristics of the 11 NUP214-ABL1–positive patients.

(TLX1 or TLX3; Table 1) compared with 41.5% (115 of 277 evaluable) in the negative group.

Clinical features of the NUP214-ABL1–positive patients

There were no significant differences in mean age (26.0 years vs 32.3 years; n = 268), sex (82% vs 75.5% male), mean leukocyte count at diagnosis (82.383/μL vs 86.081/μL; n = 258), CNS involvement (0% vs 9.8%; n = 235), therapy response (CR after induction I, 100% vs 75.9%), and, in particular, overall survival (Figure 1) between NUP214-ABL1–positive and –negative patients. 

Discussion

Several hypothetical in-frame fusion genes are possible with NUP214 (Figure 2A), and the breakpoint region in the NUP214 gene is not yet well characterized. Thus, forward primers were constructed in 10 different NUP214 exons to allow amplification of all possible NUP214-ABL1 in-frame transcripts. The ABL1 SH3 regulatory domain is encoded by ABL1 exons 2 and 3, and its fusion to another gene turns ABL1 into an oncogene. All experimentally observed ABL1 fusion genes (eg, BCR-ABL1) show a fusion of ABL1 exon 2 or occasionally exon 3 to the partner gene. Thus the reverse primer and probe in the real-time PCR were located in ABL1 exon 3. A real-time PCR format was chosen for detection because it is less susceptible than conventional multiplex PCR to misinterpretations caused by artifact bands given that it implies a sequence-specific labeled TaqMan probe.

Graux et al3 reported 4 adult patients with T-ALL with the NUP214-ABL1 fusion gene. Three died within the first year, and one had an early relapse in month 8 after diagnosis. No treatment data were given for these patients or a comparable NUP214-ABL1–negative group. The authors concluded that this fusion gene was “indicative of a rather aggressive course of the disease.” This assumption is not supported by the findings we obtained in a much larger and uniformly treated patient population. Overall survival did not differ significantly between the 2 groups (Figure 1). The major clinical features were comparable in NUP214-ABL1–positive and –negative patients (Table 1). Ballerini et al14 speculated that the presence or absence of NUP214-ABL1 in T-ALL might explain the previously reported heterogeneous clinical courses of TLX3-positive patients. The 3 TLX3- and NUP214-ABL1–positive patients in our series, however, had very different clinical courses (Table 1). We observed only 5 several possible NUP214-ABL1 transcripts (Figure 2B; Table 1). Taken together with the results of Graux et al,3 it appears that a limited number of all theoretically possible transcripts can actually be found in adult T-ALL. We therefore simplified the detection method and established a novel multiplex PCR in a real-time format.

Before the discovery of NUP214-ABL1, an involvement of NUP214 in acute leukemia was already known from AML. Here the carboxyterinal part of the gene is occasionally fused to the chromatin- and DNA-binding DEK gene on 6p23,15 resulting in a DEK-NUP214 (DEK-CAN) hybrid gene.16 The exact mode of leukemogenicity of DEK-NUP214 is unknown, but it is thought to interfere with nucleocytoplasmic transport processes.17 It is unknown whether NUP214-ABL1 also interferes with these transport processes.

A recently published case report described a NUP214-ABL1–positive patient who showed no response to imatinib treatment.18 It remains an open question whether most NUP214-ABL1–positive patients could benefit from imatinib treatment. It must also be kept in mind that processes other than NUP214-ABL1 fusion may lead to amplification of the ABL1 gene in T-ALL and to potential imatinib susceptibility.19,20

Figure 2. NUP214 and ABL1 exons. (A) In most ABL1 fusion genes (eg, BCR-ABL1), ABL1 exon 2 is fused to the partner gene, but fusion genes with ABL1 exon 3 have occasionally been described (both exons are in the same reading frame). NUP214 exons that could be joined in-frame with either ABL1 exon 2 or 3 are shaded. PCR primers were located in ABL1 exon 3 and in the 3’ part of NUP214 exons 2, 6, 10, 13, 20, 22, 26, 29, 31, and 34. (B) Agarose gel of NUP214-ABL1–positive cases. Lane 1, cell line BE-13 (transcript nup34a2); lane 2, cell line ALL-SIL (transcript nup32a2); lanes 3-13, patient samples as described in Table 1; lanes 14 and 19, eX174 size standard; lanes 15-18, multiplex PCR with H2O (lane 15), NUP214-ABL1–negative cDNA (lane 16), and positive samples (lanes 17, 18).
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Appendix

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